Undergraduate research project in the laboratory of Dr. Denis Tsygankov

A Biophysical Model of Endothelial Cell Behavior during Cerebral Cavernous Malformation.

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Introduction

Cerebral cavernous malformations (CCMs) are a collection of enlarged blood vessels with irregular structure in the brain. CCM develops in about 0.5 percent of the general population and 1.5 percent of the Hispanic population. For those with CCMs, the capillaries have a thinner and less elastic wall than normal, leading to leakage of blood vessels. Symptoms include seizures, paralysis, and sometimes hemorrhaging in the brain (Fischer et al. 2013). Currently there is no prevention and surgical treatment is often not an option. Therefore, a pharmacological method is needed to treat this disorder.

From the previous study, it was shown that CCM is majorly caused by mutation in one of CCM genes: CCM-1, -2 or -3. Particularly, disrupted tube formation was observed during the collective behavior of cells with any of these three genes mutated (Borikova et al. 2010). Since those genes play a critical role in a regulation of endothelial cell function and vascular homeostasis, loss in any of the genes would affect signaling pathways that contribute to the process of tube formation. Loss of CCM-1 gene increased VEGF (vascular endothelial growth factor) signaling which altered the organization of cytoskeletal, cell migration and cell barrier function (DiStefano et al. 2014). Also, mutation in CCM-2 increased the activation of RhoA (ras homolog gene family, member A) which led to instability in vascular homeostasis (Li 2010). Finally, it was found by Zhou et al that CCM-3 gene loss enhanced the secretion of ANGPT2 (angiopoietin 2) and caused destabilization of endothelial cell junction (Zhou et al. 2016).

Although previous research identified signaling pathways involved in the development of CCM, the biomechanics of multicellular formation in CCM is not fully understood yet. A specific relationship between cell junctions and CCM lesion development is not clear. Also, it is not clearly explained how the defects in cell interaction are translated into disease pathogenesis. Therefore, in this project, our goal is to develop an endothelial cell model to help investigate collective cell behavior in the CCM process.

Method and Result

Modeling Cell Structure

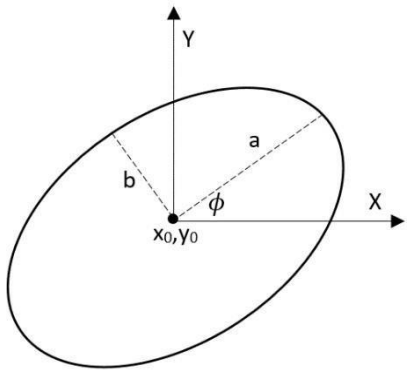


Figure 1. Parameters include Xo, Yo, a, band phi

In general, cells come in many shapes and sizes. The cell could start with simple spherical shape, but then end up with a complex amorphous shape as a result of its interaction with the environment. To allow for cell deformation, we model the cell as an extendable ellipsoid and defined its dimensions as dynamic variables, allowing the cell to change its shape under external forcing. Mathematically, an ellipse, the cross-sectional area of the ellipsoid, can be defined by X_0, Y_0, a, b where X_0 and Y_0 are the coordinates of the center (i.e. location of the ellipse), a and b are the major and minor axis, and ϕ is the orientation of the major axis with respect to the x-axis (Figure 1). When a cell interacts with the extracellular environment, the change in these variables reflects the motion and deformation of the cell. A change in X_0 and Y_0 would indicate a displacement of the cell, while a and b affect the cell's shape. A change in ϕ causes a rotation in the cell. As a first step in model development, we kept constant the variables Z_0 and c, the coordinate and cell size in the z direction. The model of the cell is shown in Figure 2. The lines coming out of the side and bottom represent the central lines of the cell protrusions, with the lengths that stochastically change over time as cells probe their environment.

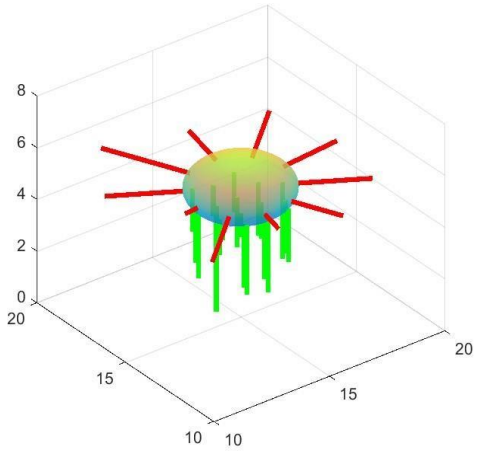


Figure 2. Cell model with protrusions involved in cell-cell (red) and cell-matrix (green) interactions

Modeling Cell Protrusion

In our model, cells move by means of protruding, attaching, and retracting protrusions to generate forces on matrix and other cells. For a cell to move, protrusions must be formed around the edges and

adhere to the surrounding area. To model this process, three key components of the dynamics were considered. First was the stochastic dynamics of cell protrusions. The change in length of protrusions was determined by a Boltzmann probability distribution equation. Two probability equations were used in the model to represent protrusion and retraction rates. The probability of each event depends on the current length of the protrusion. If the present length is smaller (larger) than a characteristic length, then the probability of extending (retracting) becomes higher. The protrusion length reaches the mean length most often but the parameter defining the width of the distribution can be adjusted to match experimentally measurable length distribution. In this setup, the protrusion persistently extends or retracts over multiple time steps before it switches the direction of motion.

The single protrusion model was used to analyze the resulting statistics and validate the performance of the stochastic simulation. Red circles in figure 3 illustrate that the protrusion length exhibit the characteristic sawtooth dynamics. Also, a histogram of the protrusion length shows that the mean length was reached the most often in the simulation (Figure 4). The reason for a high frequency of zero lengths is that negative length is not allowed in this model, therefore, defaulting to a measurement of zero. Figure 5 illustrates the behavior of the 3D model with each protrusion following the stochastic

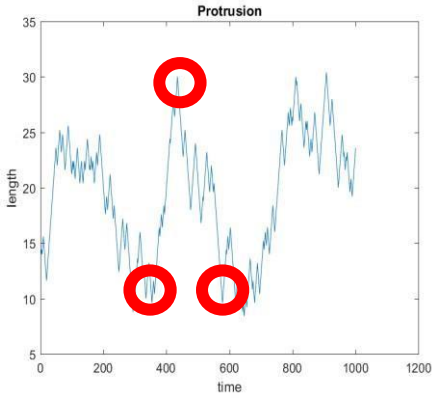


Figure 3. change in protrusion length over 50,000 simulations

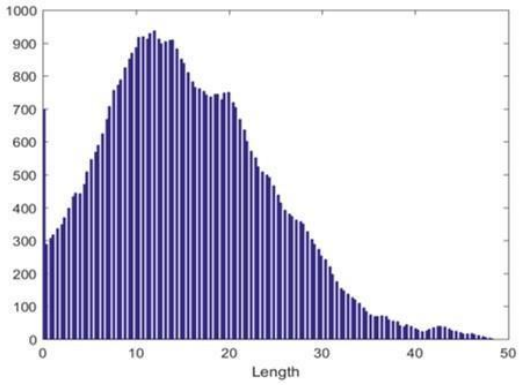
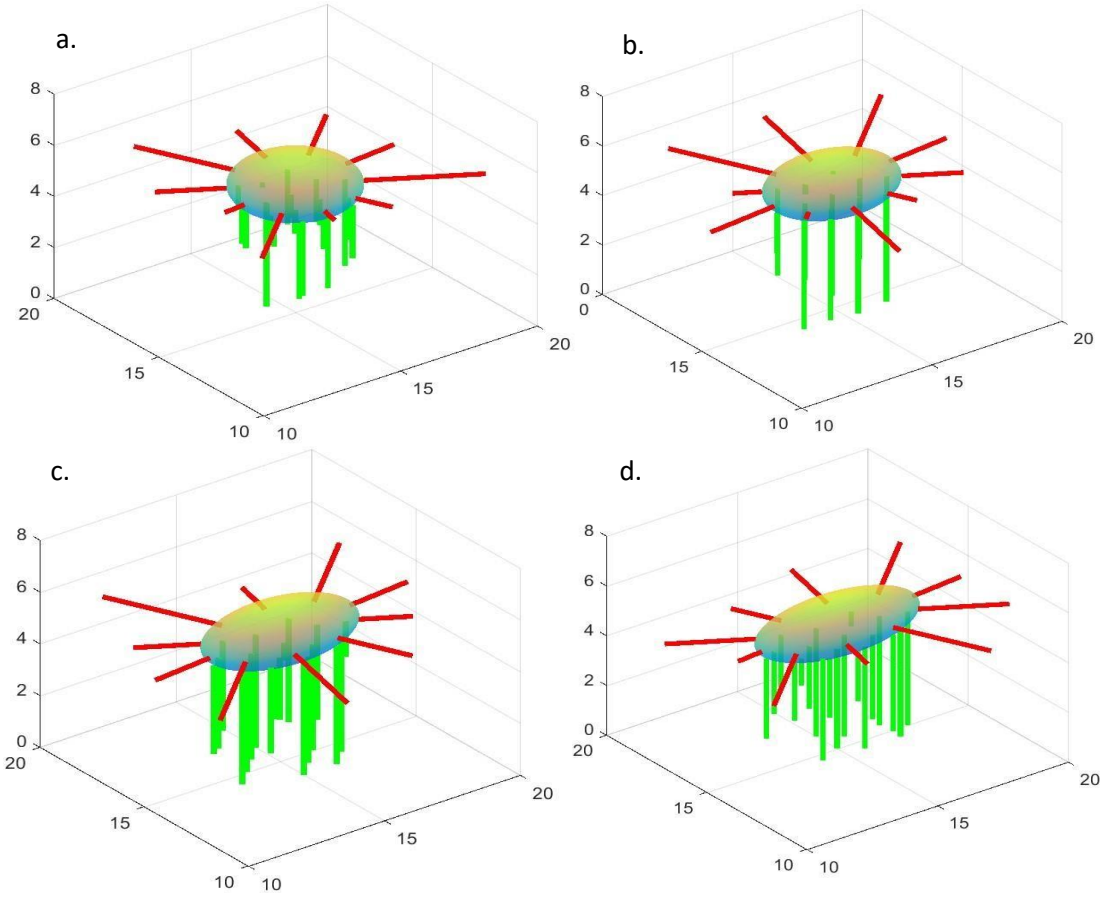


Figure 4. Histogram of protrusion length change

dynamics shown in figures 3 and 4. To illustrate that protrusion dynamics can by couples to a simultaneously change in the cell body shape, a change in cell elongation was introduced by varying parameter “a” from 2 to 3 with constant increments throughout the simulation (figure 5).



Figures 5 a-d. An illustration of the simultaneous change in protrusion lengths and the cell body shape in the 3D model.

Modeling cell interaction with the microenvironment

The next task was to model the interaction between the cell and its extracellular environment (matrix). As mentioned earlier, cells move when their protrusions attach to the matrix or another cell and contract, generating a pulling force. In our model, the movement of the cell was driven by forces calculated as the corresponding gradients of the system’s Hamiltonian, which includes the sum of potential energies from all springs representing the elastic contacts at the points of cell-cell and cellmatrix interactions. These

forces were then used to update the variable that defining the position (X_0, Y_0, and ϕ) and the shape of the cell (a, b). Therefore, the changes in the protrusion lengths collectively define cellular morphodynamics. The advantage of our approach is that the Hamiltonian of the system allows for a straightforward calculation of the changes in all variable without any complicated geometric considerations of the individual and net forces.

In addition to the previously described variables for the position and shape of the cell body, X_0, Y_0, a, b and ϕ (Z_0 and c are kept in constant for now), we introduced an additional variable ε, epsilon, to ensure the proper deformation of the cell under external forcing. The epsilon regulates the location of the protrusion bases with respect to the major axis of the cell, and like the other parameters, was updated based on the gradients of the system's Hamiltonian. As an illustration of the need for this variable, a simulation was run to compare a fix and changing epsilon as cell is pulled by a single protrusion (Figure 6 and 7). The movement of the cell upon attachment was more realistic with the addition of the variable epsilon (Figure 6) than without it (Figure 7).

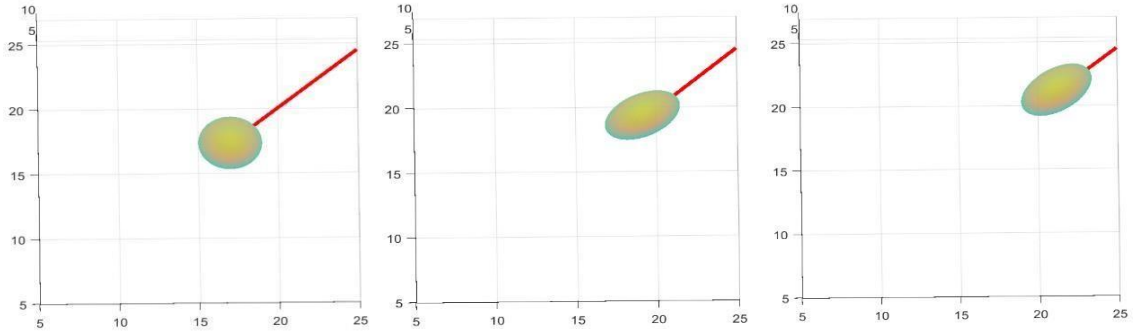


Figure 6. Cell movement with epsilon. MATLAB 2016a

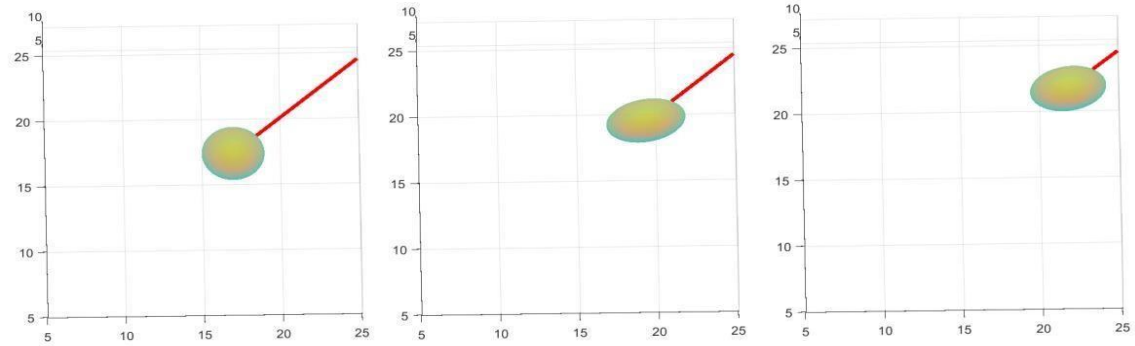
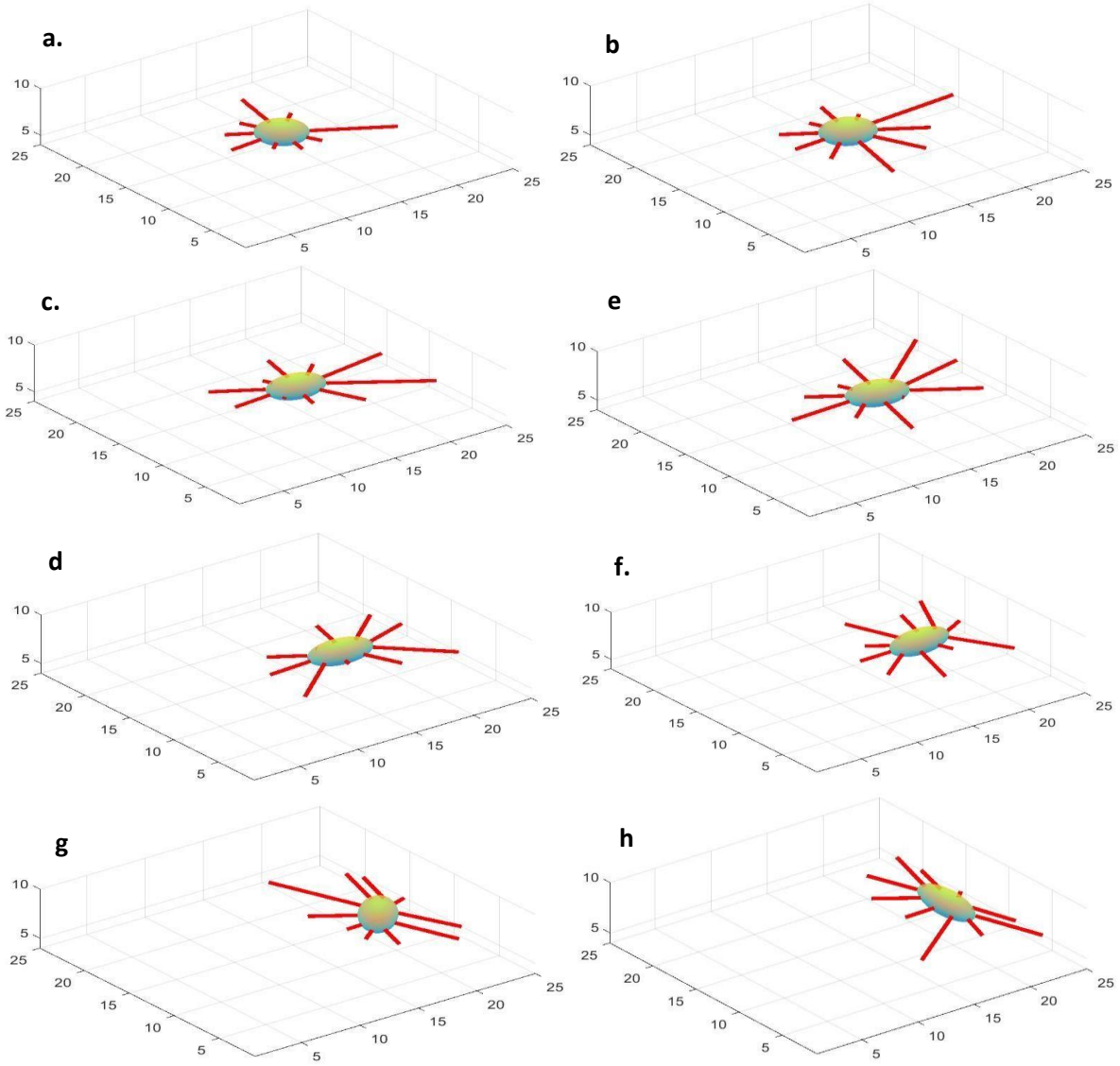


Figure 7. Cell movement without epsilon. MATLAB 2016a

In order to demonstrate the effects of the cell interaction with the environment on the resulting cell dynamics, we performed a simulation of the cell interaction with a “sticky box” (Figures 8 a-h). Once a protrusion attaches to one of the sticky box sides, the cell starts to move toward this side. At the same time, shape and orientation of the cell begins to change. Other protrusion then attaches and detaches from the box according to the stochastic dynamics of the protrusions and the stress-dependent contact breakage events.



Figures 8 a-h. Single cell behavior upon interaction with the surrounding area. MATLAB 2016a

Modeling Cell interaction between cell to cell

Our next task was to expend the model and take into account the interaction between multiple cells. Similar to the movement due to cell-matrix interaction, interacting cells move when their protrusions attach to each other and contract. In the model, the motion of a cell was driven by forces calculated as the corresponding gradients of the system's Hamiltonian, which includes the sum of potential energies from all springs representing the elastic contacts at the points of cell-cell interactions. Using these forces, the variables that define the position and the shape of the cell were updated at each simulation step. Once protrusions of one cell attach to another one, the cells start to move toward each other due to the contraction of the protrusions (Figure 9). At the beginning and middle of the simulation, the cells are significantly stretched due to the pulling forces. However, at the end, the shape recovers to the original circular form since the pulling decreases as the protrusions become fully contracted.

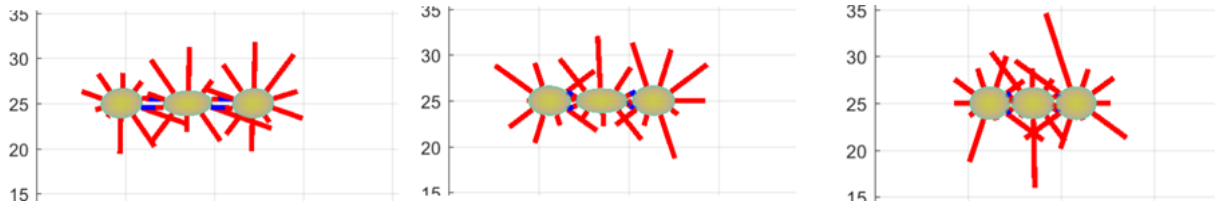


Figure 9. Cell behavior upon interaction with another cell. Blue color indicates length of the spring

Both cell-cell and cell-matrix interactions are taken into account, and a biologically meaningful behavior of the cells was reproduced (Figure 10).

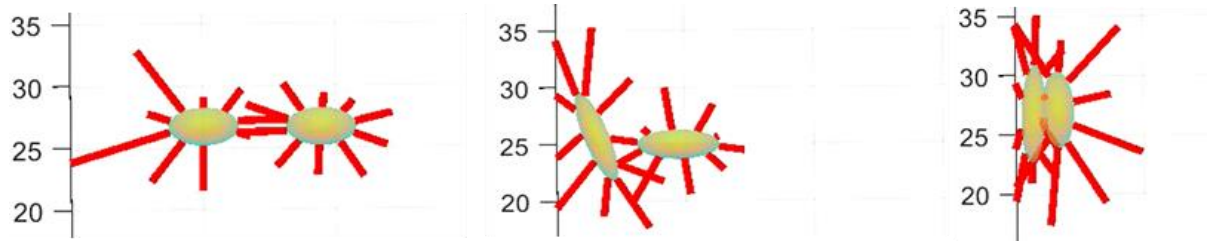


Figure 10. Cell behavior upon interaction with another cell and surrounding matrix.

Simulation of collective cell behavior

Next task was to simulate collective cell behavior of many endothelial cells plated on the surface of the Matrigel (a tube-formation assay). Before running the simulations with thousands of cells, we first performed a series of tests with a hundred cells randomly distributed over the matrix to reproduce experimentally observed behavior and optimize the parameters of the model. Testing the efficiency of simulations is a critically important step before running a realistic full-scale simulation of the tube formation process.

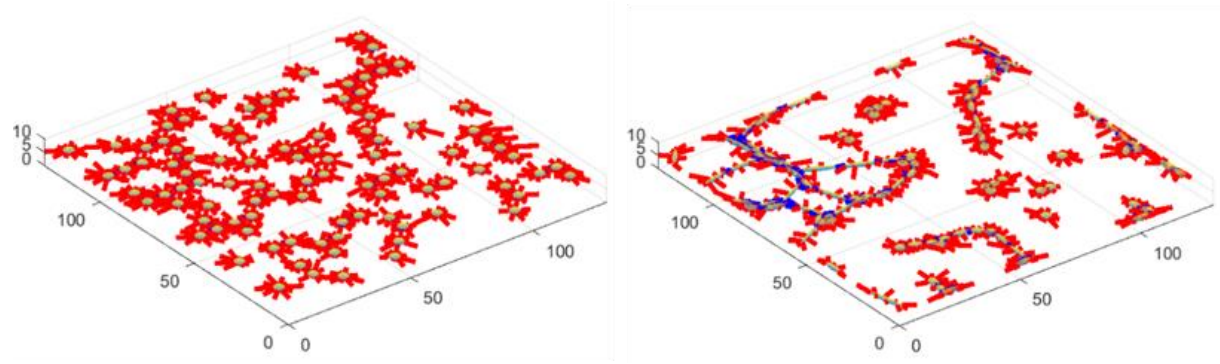


Figure 11. Collective cell behavior simulation before optimization.

An example of the simulation test with a relatively small number of cells (100) is shown in Figure 11. Although the model generates a realistic collective behavior, the simulation time did not meet our expectation and further work was required to optimize the code for a higher efficiency.

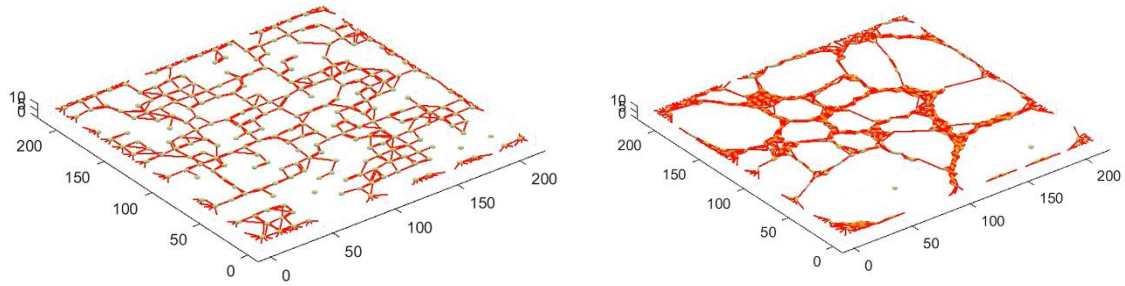


Figure 12. Simulation of collective cell behavior after optimization.

To reduce the simulation time and increase the efficiency, we used parallel computing, a processing in MATLAB which allows to carry out many calculations simultaneously. Using parallel computing, simulation time was 10 times faster than before. After the optimization, collective behavior of 300 cells on Matrigel were simulated (Figure 12). The simulation closely resembles typical tube formation pattern (a characteristic interconnected mesh of cells), which shows the consistency between the simulations and the observed cell behavior.

3-dimensional cell model with protrusions directed down

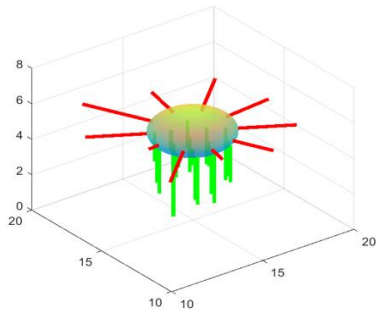


Figure 13. Endothelial cell model with sideway protrusions (red) and protrusions directed down (green).

Our next task was to take into account protrusion directed down (z-direction) in the substrate on which the cell is platted (Figure 13). Previous endothelial cell model captured the dynamics in two dimensions, accounting only sideway protrusions in a plane. Parameters responsible for z-direction (Z_0 and c) were kept fixed in the previous model. Unlike the sideway protrusions, those protrusions are responsible for the cell-ECM interaction. Our goal was to model these protrusions explicitly. When attached, these protrusions act as an anchor for cells and provide the overall stability of the multicellular formations. Therefore, it was expected that this 3D model would allow for modeling cell behavior in a more realistic way than a 2D model.

Similar to the sideway protrusions, the protrusions directed down also extend and retract in a stochastically manner. They extend and retract until they attach to ECM which triggers cellular processes making retraction to dominate over extension. However, probability of contact breakage between the protrusions to ECM was set to much higher values than the probability of detachment between cell-cell contacts on the lateral protrusions. As a result, when the protrusions attach to ECM, breakage followed soon after. Two cells with both sideway protrusions and protrusions directed down were placed in a sticky box and simulated to see their emerging behavior (Figure 14). Continuous attachment and breakage

between the protrusions and ECM resisted cell movement driven by lateral protrusions. The resulting movement depends now on an interplay between the forces generated by dynamically reorganized cell-cell and cell-ECM interactions. These forces were calculated as the corresponding gradients of the Hamiltonian which includes potential energies from all springs representing the elastic contacts at the points of cell-ECM interaction.

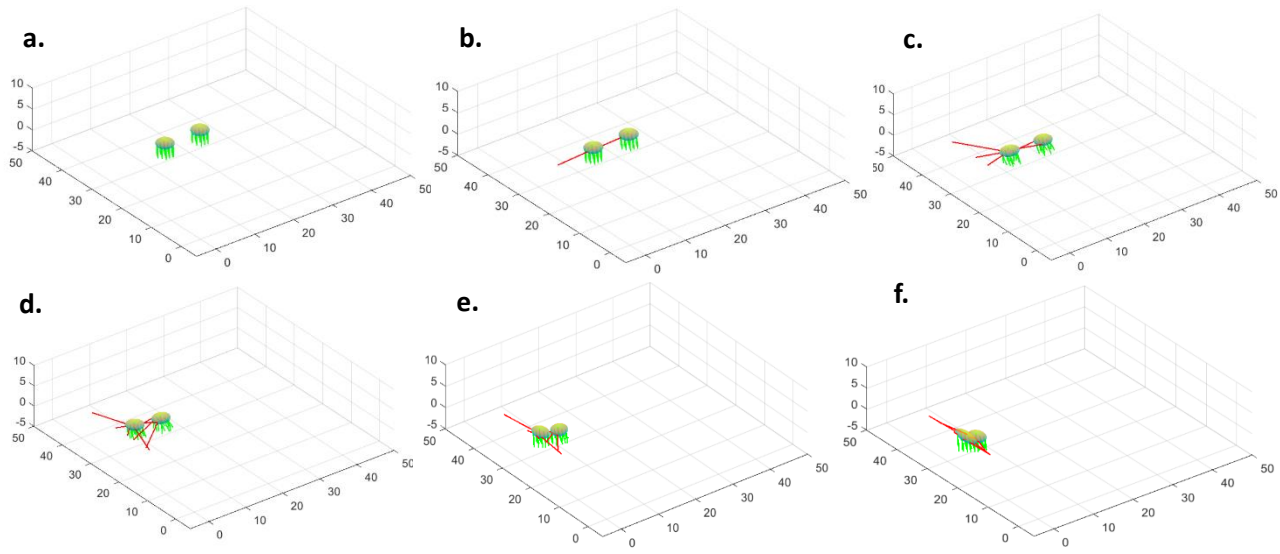


Figure 14. Cell behavior upon interaction with another cell and ECM

Conclusion/Future Work

In this project, we developed an endothelial cell model which couples with viscoelastic deformation and stochastic dynamics of the cell protrusions. Using the cell model, a collective behavior of hundreds of cells was simulated, reproducing a biologically meaningful behavior of the cells as evident from the comparison with experimental data. One big advantage of our model is that one can easily change the parameters of the model and run simulation to explore what are the parameters responsible for cellular patterns in tube formation of healthy and dysregulated cells. Also, the project has progressed towards the development of the 3-dimensional (3D) model, expecting to generate more realistic cell behavior than a 2-dimensional cell model.

The model still needs further extension and improvements. One important modification includes choosing optimal parameters to match the experimental measurement of the cell dynamics. The model contains several variables and constants that need to be fine-tuned for proper representation of the cell behavior. Thus, a series of simulations in different parameter regimes need to be performed for the validation of the model performance.

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